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William H. Reld and Antonio J. Palazzo

October 1990

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## PREFACE

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# Cold Tolerance of Plants Used for Cold-Regions Revegetation

WILLIAM H. REID AND ANTONIO J. PALAZZO

## INTRODUCTION

There are nearly a third of a million plant species. Of these, only 10–15% can tolerate any significant frost, and only a very small fraction can survive extreme cold (Dansereau 1957). For example, the arctic flora has fewer than 300 species (Polunin 1936, 1960). At 66°S Lewis (1986) found 4 mosses, 1 liverwort, 24 lichens and no vascular plants. Temperature is a major determinant of plant community structure in geographic terms (Dansereau 1957), and it explains much of the latitudinal zonation of plants (Marr 1961, Gorchakovskii and Kuvaev 1986). The inability of most species to tolerate low temperatures has a dramatic impact on many aspects of human affairs. In the Soviet Union, for example, 63% of the total land area is too cold for agriculture (Pokshishevski 1974).

The capacity of plants to survive subfreezing temperatures is called cold tolerance. Cold tolerance has several aspects: What temperatures do the tissues tolerate when the plant is dormant in the winter? What temperatures do the tissues survive in a sudden cold period when the plant is growing? Finally, and the central focus of many cold-tolerance studies, what is the minimum temperature (and the duration of that temperature) that the plant can tolerate when it has been conditioned through a natural process called cold hardening?

Cold hardening is the process by which some plant species increase their cold tolerance during exposure to low, nonfreezing temperatures. Like many engineering or agronomic terms, the phrase cold tolerance includes a condition (cold), an object subjected to the condition (plants), and a response of concern (tolerance, that is, their relative ability to survive and prosper). Thus, it reflects the intent of the agronomist to select or develop cold-tolerant plants, just as an engineer might seek a suitable steel for construction under arctic conditions.

There are several reasons why subfreezing temperatures restrict the distribution of species. The growing season may be too short to permit adequate photosynthesis for the plant to maintain a positive carbon bal-

ance. A short and cool growing season may so slow growth that the plant cannot overcome the effects of mechanical damage or grazing. However, the major factor considered in cold-tolerance studies is the effect of subfreezing temperatures on plant survival.

Even in very cold regions, periodic cold injury to plants is common (Gusta et al. 1983). Since it may be many years between record winter lows or late spring frosts, many plants intolerant of such temperatures may migrate into a region and prosper. During a cold period not all frost-sensitive plants will be killed, and soil seed reservoirs may not be damaged. Thus, the survivorship of a plant in a region does not rule out occasional significant frost damage. However, large-scale climatic differences—as with the difference in the temperate and tropical zones—does restrict the distribution of plant species.

Kinnison (1979) and Jones (1979) described and listed native and introduced plants from the Sonoran Desert of Arizona and northern Mexico damaged by one such cold spell. The giant saguaro cactus, *Carnegie gigantea*, provides an example of periodic cold injury in the Sonoran Desert of Arizona (Steenbergh and Lowe 1977). This cactus can survive a few degrees of frosts for limited periods when occasional winter outbreaks of Canadian high-pressure air reach the area. These persist for a day or more, and they damage or kill many of the cacti. The effects of chilling on various plant and biochemical functions reported by Christiansen (1978) are shown in Table 1.

In contrast, the scientific literature describing more basic research in cold tolerance often reflects a quite different focus of interest: to understand particular processes in certain species or groups of species. The ultimate scientific goal is to understand the processes and events occurring in nature. Investigators such as Sakai (1965) expose plants to temperatures far below those ordinarily encountered (–19°C and lower). This is an attempt to understanding underlying physical and physiological processes related to the cold tolerance of plants. The temperatures of significance in agronomy are higher, however, and also depend on other factors

**Table 1. Chilling effects on metabolic processes of several plant species. (From Christiansen 1975).**

Function or biochemical entity	Species	Effect of chilling
Reducing sugar	Potato	Increased
Amino acids	Bean	Increased
Protein	Bean	Decreased
Mitochondrial O <sub>2</sub> uptake	Sweet potato†	Decreased
Chlorogenic acid	Sweet potato	Increased
Oxalic acid	<i>Oxalis</i> sp.	Increased
Chlorophyll	Bean	Decreased
Organic acids	Cucurbits	Increased
ATP	Cotton	Decreased
Sugar	Cotton	Increased
Solution protein (shoot)	Cotton	Decreased
Solution nucleic acid (roots)	Cotton	Decreased
Total RNA (roots)	Cotton	Decreased
Isocitrate	Cotton	Decreased

Sweet potato, *Ipomoea batatas* (L.) Lam.

such as the location and the crop of interest. There is often difficulty in integrating literature in an area of interest to both research and applied scientists.

This review considered several issues in plant cold tolerance:

- The damage caused by freezing temperatures;
- The plant physiological mechanisms that can prevent such damage; and
- The many testing procedures for evaluating these processes.

Our objective was to identify good methodologies for evaluating and establishing species in cold environments. To this end we examined, categorized and attempted to integrate a sample of the extensive literature on cold injury and cold tolerance from many sources. Ultimately this information will aid in developing cost-effective revegetation procedures for the military and other land managers.

## TECHNICAL DISCUSSION

Like any organism, plants grow in close interaction with their immediate environment. Further, the elements of the environment that are significant to plants also interact among themselves. Evaporation, insolation (sunlight), soil-forming processes, soil microbiota, plant nutrition, and animal pollinator species and activity are a few of the many temperature-dependent variables involved in plant tolerance to cold (survival, growth and reproduction).

## Mechanical effects of freezing, ice and snow

Frozen ground, ice sheets and snow cover can damage plants through the dimensional changes of water as it freezes and thaws, the shearing effects of ice and snow on roots, and the prevention of gaseous exchange around the plant due to ice covers, resulting in the accumulation of toxic substances between the ice and the soil surface. Also, frozen soil and plant stems can prevent water translocation, which can result in the desiccation of warmer aerial plant parts.

Frost heaving has been a concern in forestry and agriculture for at least 80 years (Pearsom 1910, 1914). Several types of damage to young trees have been described (Haasis 1923, Heidmann 1976), with broken tap roots and bark slippage being the most significant. In turf-covered soils, frost heaving does not usually produce permanent damage, as the lifting usually occurs below the mass of roots. Perez (1987) described how needle ice activity controls plant distribution at 15,000-ft altitudes in Venezuela. Sigafos (1952) felt that frost action was the primary determinant of plant communities in the tundra. Smith (1987) provided a review and recent references for soil freezing processes and their effects on plants. Sayward (1979) described testing methods for studying frost heaving.

Ice sheets and snow covers that shear or slip on a vegetated surface can mechanically damage plants. Further, they may seal the surface so well that oxygen is depleted, toxic waste products such as methane and ethane accumulate, and the growth of anaerobic fungi is encouraged (Nelson and Olien 1966). In many alpine areas the presence of masses of black felt fungus on conifers provides an excellent indication of where winter snowbanks develop (Marr 1961). Problems arising from ice sheets and snow cover are usually solved by engineering control of drainage and snow deposition.

## Plant disorders produced by low temperatures

### Cell damage

Cold damage can occur in all plant organs, and several different physical processes may be involved. However, the effects of freezing within the cells of the plant is the central issue (Levitt 1978, Rajashekar et al. 1979). There are two primary cellular effects: mechanical rupture of the cell membrane and cell wall, and electrolyte imbalance as freezing removes water from solution.

### Leaf chlorosis and frost banding

In conifers, grasses and many other taxa, cold frequently induces a breakdown of chloroplasts (Treshow 1970). Leaves become a yellow-green similar to that for nitrogen deficiency. The entire leaf may turn yellow,

or the yellow will appear in bands called frost banding. Bennett (1963) also described this symptom in sugar beets as splotches or a mosaic pattern.

#### Leaf necrosis

Freezing produces a variety of necrotic manifestations in different species and with different rates of onset and duration (Treshow 1970). Part or all of the leaf discolors and dies. The area may appear wet or dry initially, and the surface may "blister" in some species. The brown or black leaves are often described as "burnt" or "scorched" by frost. In herbaceous plants, stem damage is often similar to leaf damage.

#### Albinism and malformations

Frost during the early stages of leaf development produces several special features. Marr (1947) observed aspen (*Populus tremuloides*) with albino leaves, apparently the product of a late frost that suppressed chloroplast formation and photosynthesis. Frost that damages a portion of the tissue in a growing leaf can result in a variety of geometric distortions as the still-living cells continue to divide. Examples of malformations include stem cracks in trees caused by tension as

the trunk is rapidly cooled (Beal 1962, Bervaes et al. 1977). In subsequent years a cavity may form in the injured areas; this is called blister shake (Tyron and True 1952) and boll canker (Barnard and Ward 1965).

When the cambium is killed by freezing, the symptom of "top drying" may result. This usually occurs during a cold period after an exceptional winter or spring warmth, but it may not be visible until later in the year as the needles brown.

#### Hardening and induction

Research has demonstrated that cold hardening is a plant property that must be induced by suitable environmental conditions. Only those plants with a genetic capacity to harden can express this acclimation. There is universal agreement that the acclimation develops during a period in which the plant is exposed to low, nonfreezing temperatures and shorter days (Andrews 1960, Krasavzev 1961, Chen et al. 1977, Chen and Li 1978, 1980, Gregory and Poff 1979). Smith (1975) reported on studies in Wisconsin measuring the decline in electrical conductance or the increase in cold resistance of plants that begins to develop in the fall (Fig. 1). Without hardening, cells easily freeze and mem-

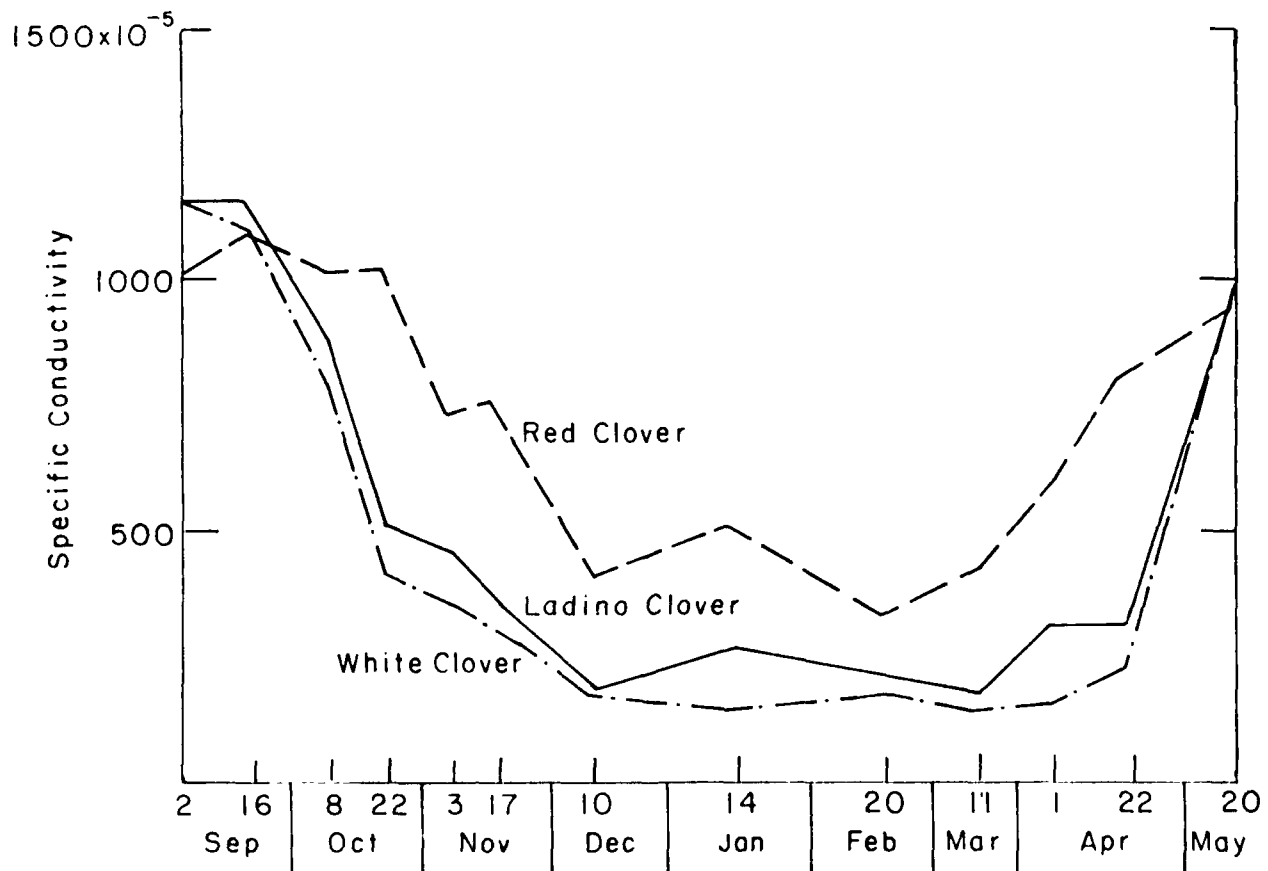


Figure 1. Development of seasonal cold resistance in plants. (After Smith 1975.)

branes are torn by ice crystals (Pukacki and Pukacka 1987, Andrews 1987).

The capacity to develop cold tolerance by appropriate hardening or induction is a seasonal occurrence (Sakai 1966, Sakai and Otsuka 1970, Stushnoff and Junttila 1986). As a general rule, plant species that can cold harden will do so with 10–14 days of exposure to temperatures below 6°C. Hardening is easily lost during vigorous growth produced by brief warm periods (Andrews 1960, Kaku 1971b, Sakai and Otsuka 1970). Grasses can develop cold tolerance in the earliest seedling stage (Cohen 1980, Cohen and Wood 1986, Wood and Cohen 1986).

Physiologically, cold hardening is a cellular process (Dexter et al. 1930, 1932, Dexter 1933, 1956, Samygin 1987). Early evidence came from electrolytes released from ruptured cells, which could be detected by changes in the electrical conductivity of fluid extracts. Gregory and Poff (1979) provided conclusive evidence for the cellular basis of cold tolerance by producing cold-tolerant cells in loose suspensions.

The genetic influence of the hardening process varies among species, subspecies and varieties or cultivars (Parker 1960, Smithberg and Weiser 1968, George et al. 1974, Khantimer 1974), and cold-hardy cultivars can be improved with selective breeding (Hayes and Aamodt 1927, Youngner and Nudge 1968, Fowler et al. 1977, Hurley and Funk 1983, Flander 1984, Fennell and Li 1987). Most of the genetic research concerns wheat. Major papers include Hayes and Aamodt (1927), Law and Jenkins (1970), Cahalan and Law (1979), Sutka (1981) and Sutka and Kovacs (1985).

Efforts to transfer cold-tolerance expressive genes has had only limited success. Limin et al. (1985) attempted to transfer the rye cold-tolerance genome to wheat in hybrids. While the gene transfer was successful, its expression was entirely suppressed in the offspring. Roberts (1986) concluded that several loci on different chromosomes of wheat were invoked by different hardening regimes. Consequently, selection processes using different temperatures yielded different degrees of hardness. New techniques in molecular genetics will likely improve our understanding of this nontranslocatable, cellular process.

### Carbohydrates

Carbohydrates are central to the plant's physiology, structure and function (Devlin and Witham 1983). The carbohydrate economy of both cultivated and natural plants is crucial to their capacity to survive and reproduce (Mooney 1958). An important process in cold hardening is the accumulation of soluble carbohydrates within cells during hardening (Mooney 1958, Parker 1962, Trunova 1963, Okajima and Smith 1964, Sakai

and Yoshida 1968, Levitt 1969, Dunn and Nelson 1974, Pratt and Smith 1982, Fege and Brown 1984, Gunn and Walton 1985, Belding and Young 1987). Thus, an increase in these carbohydrates after hardening is evidence by which to screen plants for cold-regions revegetation applications.

Soluble carbohydrates depress freezing points and prevent ice formation within plant cells (Okajima and Smith 1964, Nelson and Smith 1969, Dunn and Nelson 1974, Moser et al. 1982, Andrews et al. 1984, Belding and Young 1987). Research on the nature of the different sugars accumulating in plant tissues began over 40 years ago (Nelson 1944), and the significance of each is still being investigated (Trunova 1963, Moser et al. 1982).

Carbohydrate accumulation in cold-hardened plant tissue may account for a highly significant fraction of the plant's weight. Gunn and Walton (1985) reported that a winter-green tussock grass of South Georgia and the Falkland Islands accumulates as much as 71% by weight of nonstructural carbohydrates in its stems in winter. Younger and Nudge (1968, 1976) reported that carbohydrate accumulation in Kentucky bluegrass is temperature dependent.

Although cold hardening has a genetic basis and is partly the result of carbohydrate accumulation, other factors contribute to cold tolerance. Plant water status has a largely independent effect. Chen et al. (1977) found that seven days of water stress increased the cold hardness of *Cornus stolonifera* from -3° to -11°C. Hurst et al. (1985) found a loss of total available carbohydrates with prolonged wetting (Fig. 2).

The bulk of opinion is that drought-induced cold tolerance is a passive process deriving its effect from increased concentrations of solutes in cells and extracellular spaces (Palta et al. 1979). However, Sakai (1978, 1979) found that water migrated from bud tissues during slow cooling. This produced needle ice crystals beneath the crown of the shoot but reduced ice formation within the tissue. Whatever its origin, this mechanism increased the bud cold tolerance.

Plant nutrition also affects cold tolerance. Most notably, good nitrogen nutrition at certain times decreases cold tolerance (Carroll and Welton 1939, Parks and Fisher 1958, Adams and Twersky 1960, Jung and Kocher 1974, Helm et al. 1987). In contrast, good phosphorous and potassium nutrition improves cold tolerance (Adams and Twersky 1960). The reason for the effects of phosphorous and potassium are unknown, but there is general agreement that nitrogen induces the growth of new, water-filled cells with low carbohydrate concentrations and greater sensitivity to cold.

The status of several cellular chemical groups changes with the development of cold tolerance. The most

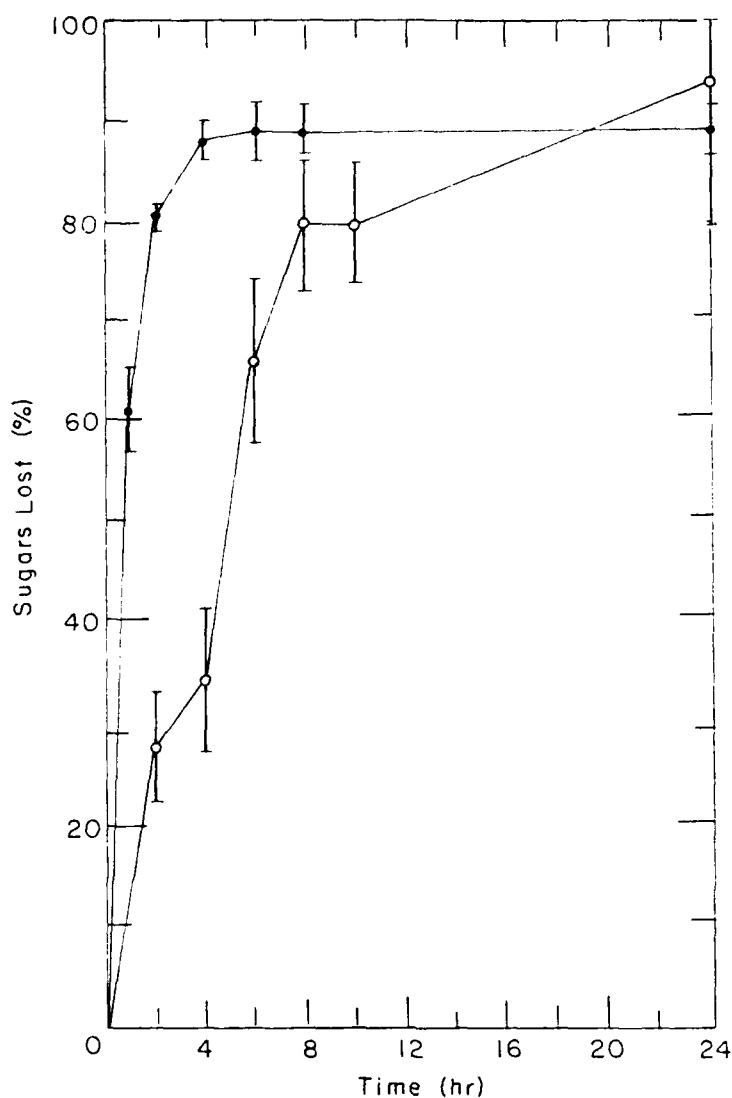


Figure 2. Effect of prolonged wetting on the loss of reducing sugars from mature-senescent leaves of *Poa flabellata* (●) and *Acaena magellanica* (○) expressed as the percentage of total reducing sugars, with standard deviations. (After Hurst et al. 1985.)

striking of these, as discussed above, are the soluble, nonstructural carbohydrates. However, cellular lipids and proteins also increase (Siminovitch et al. 1968), largely as a result of the proliferation of intracellular membranes during cold hardening. These lower the freezing point of cell fluids. Siminovitch et al. (1968), in one of the few studies of cellular anatomy during cold hardening, obtained evidence suggesting that these membranes compartmentalize the cell. The compartments may inhibit ice crystal formation or at least prevent ice crystal growth. Bliss (1962) found very high lipid levels in cold-adapted alpine tundra species. In alfalfa, Kuiper (1970) found phosphatidyl glycerol, phosphatidyl inositol and sulpholipid content to in-

crease at lower growing temperatures. He also found significant differences in other lipids between hardy and sensitive varieties of alfalfa. Siminovitch et al. (1968) and Novitskaya et al. (1987) found an increase in lipids associated with membranes and a decrease in lipids such as sterols. An analysis of data for two grasses grown at different temperatures (McCown 1973) shows the total lipid content to increase with increasing carbohydrates. Dipaola (1979) reported a similar increase in the fatty acid content of turfgrasses.

Cold tolerance is also reduced by any activity that results in lower cellular carbohydrate concentrations, such as increased growth or management practices. Wood (1986) found both increased cold damage and

lower carbohydrates in pecan trees that had produced large nut crops during the season. Andrews et al. (1984) found that winter wheat cut in the fall had lower levels of nonstructural carbohydrates and slightly reduced cold hardiness and ice tolerance. Grierson and Soule (1979) suggested that it is important to test a combination of factors that affect cold tolerance in plants. Certainly this is true for revegetation problems where mechanical and other stress will be present during regrowth.

A number of chemicals have been found to alter plant cold tolerance. Li and Weiser (1973) artificially enhanced short-term cold tolerance by applying cysteine along with a two-week period of short-day induction. Untreated plants died at  $-5^{\circ}\text{C}$ ; treated plants survived to  $-11^{\circ}\text{C}$ . Brown (1974) used growth-retardant chemicals to increase cold tolerance. Zhang et al. (1987) found that meliudide reduced chilling injury in rice seedlings by reducing electrolyte leakage through cell membranes.

The rate of carbon fixation through photosynthesis is an important background issue in the arena of plant survival in cold climates and appears to deserve further study of its relationship to cold tolerance. The rate of photosynthesis varies with temperature (Hodgson et al. 1987); photon flux density, duration and spectral properties; and plant genetics and nutrition. The issue of significance is the carbon balance of the plant, since it must have a positive carbon balance to grow, reproduce and accumulate adequate carbohydrates in the tissues. Recent analyses and simulations permit this evaluation (Schmidt and Blaser 1967, Penning de Vries et al. 1974, Hesketh and Jones 1976, Thornley 1976, 1977, Brockington 1978, de Wit 1979, Hunt and Loomis 1979, McCree 1982). To date, these techniques have not been used in evaluating plant cold tolerance in different environments.

The different photosynthetic pathways used by plants has some importance in understanding carbon balance and cold tolerance. Devlin and Witham (1983) provided a summary of the photosynthetic process. The most common pathway in temperate and boreal species is the Calvin-Benson (or C3) pathway. Some plants, particularly warm-region species, use a process producing four-carbon compounds (the C4 or Hatch-Slack pathway). These also have a special vascular anatomy called Kranz. Some succulents have the C4 pathway but not the Kranz anatomy. These fix carbon dioxide as malic acid and are known as crassulacian acid metabolism (CAM) species. Many species switch pathways as environmental conditions change.

There has been little study of C4 and CAM plants as revegetation species for cold environments. *Agave*, a traditional food source in the Southwestern deserts, has very large carbohydrate reserves and is cold tolerant

(Reid, unpubl. data). Other desert succulents such as *Yucca* and *Dasylirion* are also cold tolerant. *Sedum*, a succulent genus with species growing in the alpine and subalpine zones (Marr 1961), is also cold tolerant.

### Test methods

Testing of cold tolerance and cold hardening falls in four general areas: measurements of biochemical and morphological features that are correlated with cold tolerance; laboratory and phytotron tests; field trials; and measurements of cold damage by either qualitative or quantitative means. Here we discuss a few of the more important techniques.

#### *Colorimetric carbohydrate determination*

Determination of soluble carbohydrate concentrations in tissues has been the primary technique for evaluating potential cold tolerance. The origin of the method was in the chemical-colorimetric determination of sugars in blood and urine (Roe 1934, Nelson 1944). Applications in agriculture then led to a number of variations as the spectrum of carbohydrates—particularly various hexose polymers—increased (Weinmann 1947, DuBois et al. 1956, Johnson et al. 1964, Smith et al. 1964, Van Handel 1967, 1968, Smith 1969, Van Handel et al. 1972, Gaines 1973, Southwick et al. 1981, Moser et al. 1982, Westhafer et al. 1982).

#### *High-performance liquid chromatography (HPLC)*

HPLC allows rapid and precise determination of nonstructural carbohydrates. Some of the recent work using HPLC for carbohydrate determination is by Wilson et al. (1981), Barton et al. (1982), Ford and Howse (1982), Freeman et al. (1983, 1984, 1985), Dahlqvist and Nilsson (1984), Praznik et al. (1984), Picha (1985, 1987) and Reid and Freeman (1985). Available electronic data acquisition systems speed and simplify operations. Some initial applications used ultraviolet detection (195 and 254 nm), but refractive index detectors are now used.

The HPLC method of Wilson et al. (1981) for fructose, glucose and sucrose is summarized here since the process is not widely known. Two columns were used. The first removed residual solids, and the second was one of a number available to separate carbohydrates. The mobile phase was acetonitrile-water (75:25) at 1.8 mL/minute. Plant fluids were extracted. Proteins were precipitated with methanol and then centrifuged, and the supernatant passed through a filter. Then 6–20 mL of the fluid were injected into the chromatograph. The instrument was calibrated by injecting known masses of different sugars.

With nearly pure sugar solutions such as floral nectars, sample processing is not necessary. These may be

injected directly after removal from the plant. Nectars ordinarily have a total sugar content of 15–25%, and samples as small as 1  $\mu$ L can be used for determining sugar composition (Freeman 1982, Reid and Freeman 1985). Both these techniques indicate that sugar masses as low as 0.1  $\mu$ g can be detected and measured with precision by HPLC.

#### *Visual detection of cold damage*

Cold-induced plant damage is frequently detected and measured by visual inspection by trained investigators. For example, the very large study of Adams and Twersky (1960) relied on appraisal by three judges who ranked 192 grass stands. More sophisticated efforts involve measuring (or estimating) cover, height and production. Production is measured by clipping or mowing selected strips or quadrants (Hayes and Aamodt 1927, Andrews 1960, Jung and Kocher 1974, Fowler et al. 1977, Flander 1984, Burns et al. 1985).

#### *Tissue and fluid electrical conductivity*

A number of early investigators have used changes in plant electrical resistance or conductivity to detect frost damage (Dexter et al. 1930, 1932, Dexter 1933, 1956, Greenham 1966, Ketchie et al. 1972, Samygin 1987). Hayden et al. (1969) and Stout et al. (1987) used more sophisticated measurements of both resistance and impedance at different applied frequencies.

#### *Chlorophyll fluorescence*

MacRae et al. (1963), Smillie et al. (1987) and others have used chlorophyll fluorescence to detect degenerative changes in the cell as fluids escape after freezing.

#### *Nuclear magnetic resonance (NMR)*

NMR has been used to measure the quantity of frozen water in plant tissue (Chen and Gusta 1978, Chen et al. 1976, Palazzo et al. 1984). This method may be used to correlate the amount of ice with temperature, which then may be correlated to plant injury or death.

#### *Freezing exotherms*

Kaku and Salt (1968), Kaku (1971a,b, 1973, 1975), Kaku et al. (1981) and Sakai (1978, 1979) have led in studies of exotherms in subcooled tissues as indicators of freezing in woody plant tissue. Paired live and dried plant tissue samples are connected by thermocouples in series and slowly cooled. Freezing in the live tissues releases heat detectable as a small, sudden change in thermocouple electrical potential. After cold exposure, plants are grown in pots to demonstrate viability. Wolf and Pool (1986, 1987) applied these techniques to grapevine buds, analyzing the exotherms by direct input to a minicomputer.

While the exotherms do not pinpoint the location of tissue water, some useful interpretations can be made. For example, fruits of the barrel or fishhook cactus, *Ferocactus wislizenii*, show a large initial exotherm at  $-6^{\circ}\text{C}$ , no further exotherms when cooled to  $-30^{\circ}\text{C}$ , and no damage when thawed. Fruit later re-cooled to  $-45^{\circ}\text{C}$  showed that a series of small exotherms occurred at  $-32^{\circ}$  to  $-36^{\circ}\text{C}$ . After this second treatment the fruit softened immediately on thawing (Reid, unpubl. data). These data suggest that the first exotherm was extracellular water freezing, producing no damage. The low-temperature exotherms in the second test were cellular and damaged the fruit. In nature the fruit of this cactus remains on the plant during the winter, often exposed to  $-10^{\circ}$  or  $-20^{\circ}\text{C}$ , and it matures in the early spring. The freezing exotherm data are consistent with this reproductive habit.

## **BASIC AND APPLIED RESEARCH NEEDS**

The U. S. Army needs vegetation information for tactical and environmental reasons. For tactical purposes, plants provide data on previous land use in areas with troop movements and chemical munitions use. This information may be gathered on the ground or by remote sensing. Vegetation is also required to maintain land for training missions. Today's training technology requires greater land areas for the same number of troops. When vegetation fails in training areas, the utility of the site will be reduced. For example, deep erosion gullies may form, dissecting the training areas into small, less useful sites.

The National Environmental Policy Act, laws implementing its provisions, and state and national regulations create a need for cost-effective revegetation techniques applicable to large areas of terrain. Army lands may be subjected to stresses and contaminants not earlier considered by agronomists, and these new situations create a need for additional research, particularly if cost-effective methods are to be found for maintaining these lands.

Studies in several areas will dramatically improve our understanding of plant cold tolerance and capacity to revegetate cold regions:

- Basic research on the fundamental aspects of the physiology, molecular biology and biophysics of cold tolerance;
- Research on cold tolerance in the presence of other stresses common in field situations;
- Quantification of climatic factors and biological responses, permitting better revegetation planning;
- Applied research on effective field and remote

sensing methods for monitoring vegetative status; and

- Applied research on large-scale revegetation to find minimum-cost solutions.

Some specific topics within these broad areas are as follows.

#### **Fundamental processes**

While the role of carbohydrates is known, there are data on only a few species. Broad-ranging studies with modern techniques can provide a survey of different species and can determine carbohydrate changes with different soil and nutrition conditions and with time, as well as the effects of specific sugars. In grasses, for example, sugar ratios are different in the leaves, stems and floral structures (Palazzo, unpubl. data). These ratios seem to change with the carbohydrate accumulation of cold hardening.

A fundamental understanding of cold hardening and tolerance is important. Further, laboratory equipment, such as the high-performance liquid chromatograph (HPLC) supported by a minicomputer, permit a speed and precision far greater than has been possible in the past. Nuclear magnetic resonance (NMR) permits a better understanding of cellular freezing (Chen et al. 1977, Palazzo et al. 1984).

The plant microanatomical changes associated with cold hardening have received little study. It is probable that work with modern techniques, including transmission and scanning electron microscopy, will yield important insights.

Cells of some plants can reach  $-60^{\circ}\text{C}$ , and even  $-250^{\circ}\text{C}$ , without damage. The physical state of the intracellular water at these temperatures is not well understood. That the bulk of water is in some ordered form is reasonable, but how lethal electrolyte concentration changes are avoided is unknown.

#### **Cold mechanical damage and amendments**

A number of factors affect plant survival. Revegetation is often done in areas subjected to several stresses. Further, it may be that sludge treatment of soils will prove to be cost effective for improving plant growth. Sludge is an excellent soil conditioner and fertilizer that is often available at low cost. However, the nitrogen content of sludge may reduce the winter survival of traditional revegetation species if applied at excessive rates. Burns et al. (1985), for example, found that swine lagoon effluent applied to coastal bermudagrass greatly increased winterkill at a study site in North Carolina. While this was not a revegetation site, it illustrates a problem that might be avoided by adequate research and

field testing. Additional research is needed on the combined effects of cold and mechanical stresses, including foot traffic and wheeled and tracked vehicle movement.

#### **Climatic factors**

Several climatic changes are significant to plants. These include

- The pattern of fall cooling. When does the first freeze occur relative to shortening days? How long do cooler days allow the plant to harden before the first freeze? How cold is the first freeze?
- The extreme of cold and its duration.
- Midwinter thaws or chinook winds.
- The pattern of spring warming. Are freezes likely after growth is initiated and hardiness lost? Expression of the very large national weather data base in terms significant to plant survival would improve our capacity to plan revegetation effectively.

Many agronomists have developed cold-hardy plant varieties using winter temperatures as the selective factor in open field trials. However, there is much year-to-year climatic variation that confuses results. This led Dexter et al. (1930) to develop the use of environmental chambers and tissue conductivity measurement to standardize selection and to better quantify hardiness. Further, the significance of temperature is coupled with factors including wind, humidity and precipitation.

Modeling is a logical step in the standardization of methods. One could plan revegetation considering the severity of several factors, and design for a 20-year winter as one now speaks of floods. A land manager could then use cheaper short-term strategies where other physical damage is frequent, and different methods where long-term plant survival is of utility.

#### **Simulation analysis**

Geiger (1957) observed that net thermal radiation can result in plant and soil temperatures much lower than the ambient air temperature. Quantitative models could provide insights of importance to revegetation. With modern computing capacities it is possible to simulate a grass turf or tussocks and other plant forms, and find leaf and soil temperatures for typical extreme nights. The common arctic growth forms of tussocks and rosettes could be compared with those of other species.

#### **Plant growth models**

Even if a plant has adequate cold tolerance, it must also be able to grow and reproduce. It must have a net

positive carbon balance during most years. This can be determined by long-term testing or, with increasing precision, by simulation or modeling. Models simulating plant physiology and environmental conditions are possible. A growing number of studies suggest this is a practical step in cold-regions revegetation studies (Hesketh and Jones 1976, Thornley 1976, 1977, Penning de Vries et al. 1977, Brockington 1978, de Wit et al. 1979, Hunt and Loomis 1979, McCree 1982).

#### **Remote evaluation of damage and revegetation success**

Both initial studies of damage and later evaluation of revegetation can be expensive on large ranges. Further, these techniques are often qualitative or subjective. Their success has depended largely on the skill of trained agronomists. The use of remote sensing techniques offers a nearly developed potential for ongoing monitoring of a number of sites from central facilities. Airborne or spacecraft imaging and spectral analysis systems (airborne imaging spectrometer, synthetic aperture radar, etc.) have the potential for an automated analysis when couple with a geographic information system (GRASS or GIS). A development program coordinated with revegetation programs could lead to cost reduction as the area under management increases.

#### **Nontraditional species and strategies**

Most cold tolerance and revegetation research has used a rather small set of species from mesic environments in the temperate or boreal zones. Many other species are available in nature; Grierson and Soule (1979) suggested that drought-tolerant species are candidates for cold tolerance. The xeric conditions and poor soils of the western United States support species that prosper and provide good animal forage. The grama (*Bouteloua*) and buffalo (*Buchloe*) grasses of the short-grass prairie grow well with 10–15 inches (25–38 cm) of precipitation and endure winter temperatures below –30°C. Ecologists and agronomists have begun to examine the role of natural plant succession and soil-forming processes as guides to revegetation and land management (Arnalds 1987, Webber and Fridriksson 1987). Continuing research is needed.

#### **Soil microbiology**

A peripheral, but important, issue is research on cold-regions soils (Sayward 1979). Knowledge of soil microbiology in cold regions is even more limited (Parinkina 1971, 1972, 1985). However, an appropriate soil microflora is essential to many plants (Treshow 1970). Indeed, pines, lacking root hairs, depend on soil fungi for water and mineral nutrition. They cannot

survive without these fungi. As cold-regions revegetation becomes more sophisticated, additional research in soil microbiology will become essential.

#### **Information resources for research and the land manager**

There is a need for a central clearing house of biological cold-tolerance and cold-regions revegetation data. The USA, the USSR, Canada, the UK, Japan, Iceland and the Scandinavian countries are among those involved in cold-regions revegetation research. For example, a brief and incomplete review of the Russian literature showed the following as a sample: Akul'shina et al. (1979) examined the revegetation of lands on tundra petroleum pipeline terraces. Kolesnikov (1974), Kolesnikov and Motorina (1978) and Motorina (1978) examined this same issue for "technogenic landscapes." Kuz'min (1985) tested methods of recultivation of tailings using lakebottom silt over drillrig tailings. Skryabin (1979, 1980) reported on revegetation in tundra. A central data source for both journal contributions and technical reports would make a significant improvement by speeding research and helping avoid redundant studies.

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13. ABSTRACT (Maximum 200 words)  Only a fraction of the world's plant species can tolerate freezing, and all exhibit various forms of damage after exposure to extreme cold. Some species, on exposure to low, nonfreezing temperatures, exhibit enhanced tolerance through a genetically determined process called cold hardening. Cold tolerance is attributed partly to the accumulation of soluble carbohydrates, soluble proteins and lipids in cells, and to the proliferation of intracellular membranes. There are several methods of testing for cold tolerance. Plant nutritional status may increase or decrease cold tolerance. Several chemicals, among them a fungicide, have been found to reduce cold tolerance. Water stress improves cold tolerance. Research is needed in several areas to improve the success and lower the cost of revegetation projects. The genetics of cold tolerance is poorly understood. Research on cold tolerance with combined stresses is needed. Simulation analysis of plant growth in cold climates is important if carbon balance is to be understood. Applied research is needed in several areas: appropriate statistical descriptions of climate, remote sensing for terrain evaluation, analysis to determine plant and soil temperatures in relation to air temperature; complex revegetation strategies involving plant succession on disturbed lands. Cold-regions soil microbiology, important in plant success, is poorly known. A clearing house for information on plant cold tolerance and cold-regions revegetation would reap great reward for efficient reclamation. *					
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